15

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YELLOW FEVER WITHOUT *AËDES AEGYPTI*. STUDY OF A RURAL EPIDEMIC IN THE VALLE DO CHANAAN, ESPIRITO SANTO, BRAZIL, 1932.*

BY

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Introduction.

Following the demonstration by the American Army Commission of the transmission of yellow fever by *Aëdes aegypti* (Linnaeus), these investigators and other workers carried out a limited number of experiments in which other species of mosquitoes were tested as possible vectors of the disease. These experiments gave uniformly negative results. The necessity of using human volunteers in this early work prevented a thorough canvass of the field, and it was not until more than a quarter of a century later that successful inoculation of animals with yellow fever virus led to the testing of a number of different mosquitoes. Bauer (1) and Philip (2, 3, 4, 5) in Africa, and Davis and Shannon (6, 7, 8) in South America have shown that several species of mosquitoes may transmit the virus from animal to animal in the laboratory and that other species not yet shown to transmit the virus may harbor it in a living state for a period equal to the usual incubation period in *Aëdes aegypti*. Wide variations in facility and regularity of transmission have been reported for the different species studied.

Among epidemiologists and students of yellow fever the disease has long been considered essentially an urban one, with *Aëdes aegypti* necessary for its transmission in nature (Carter, 10). The dramatic disappearance of yellow fever from all the notorious centers of distribution on the American continent, following the application of measures directed against the reproduction of *Aëdes aegypti*, seemed to confirm

* The studies and observations on which this paper is based were conducted by the Cooperative Yellow Fever Service maintained by the Brazilian Government and The Rockefeller Foundation, with the active participation of the Health Department of the State of Espirito Santo, Brazil, and the Yellow Fever Laboratory of The Rockefeller Foundation at Bahia, Brazil.

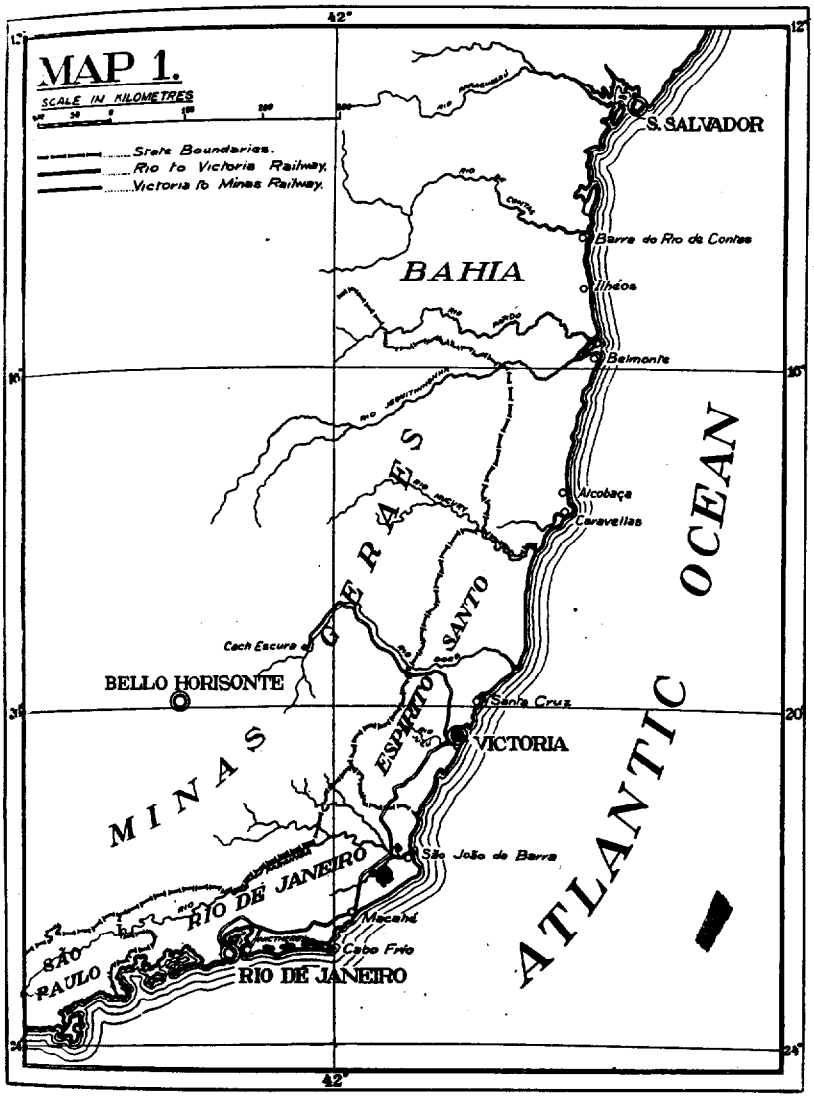
the belief that this species was the only one of importance in the production of natural infection. Yellow fever has never failed to disappear from the morbidity and mortality records of cities and towns shortly after the reduction of *Aëdes aegypti* breeding, and the absence of this species from a locality was often accepted as sufficient evidence that yellow fever did not exist there.

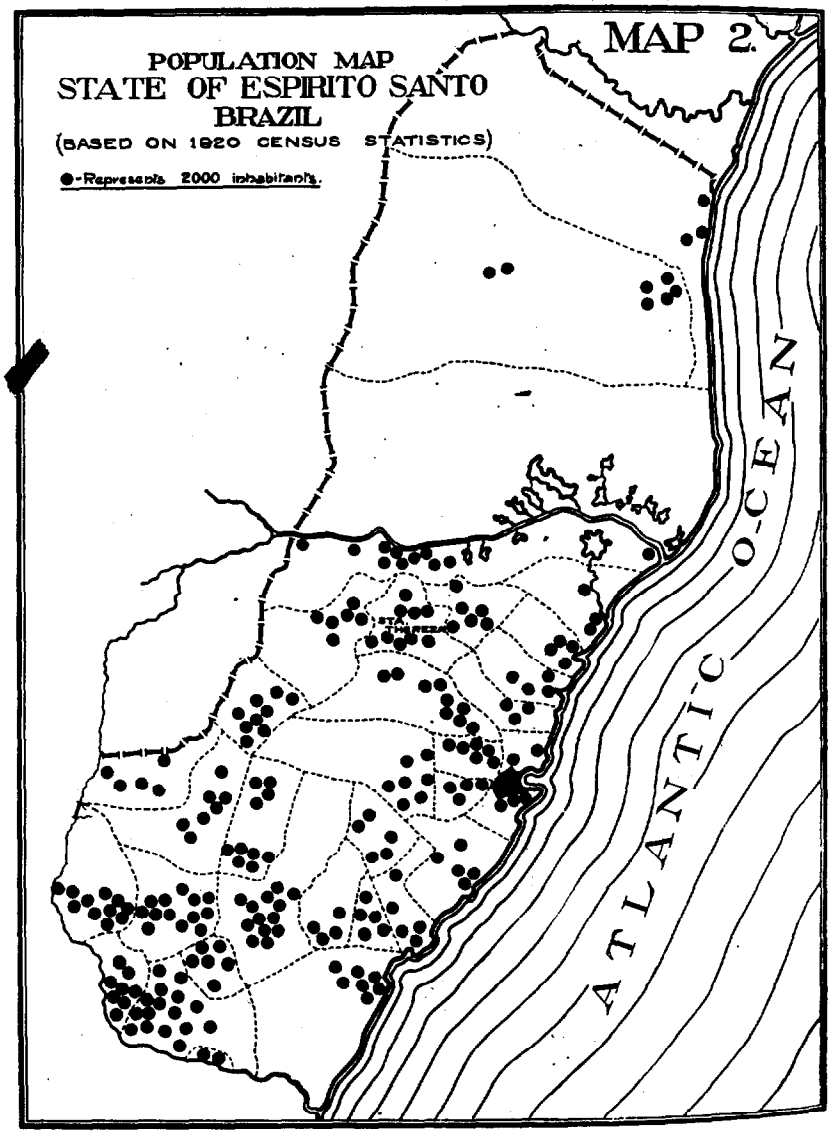
Beginning in April, 1930, an attempt was made to determine the distribution of yellow fever in Brazil, through the routine histological examination of liver specimens from persons who had had fatal febrile infections of less than ten days' duration. This attempt resulted in the diagnosis of yellow fever in certain isolated cases in rural areas where subsequent field investigations failed to reveal other cases of the disease, and suggested that *Aëdes aegypti* probably could not have been responsible for transmission.

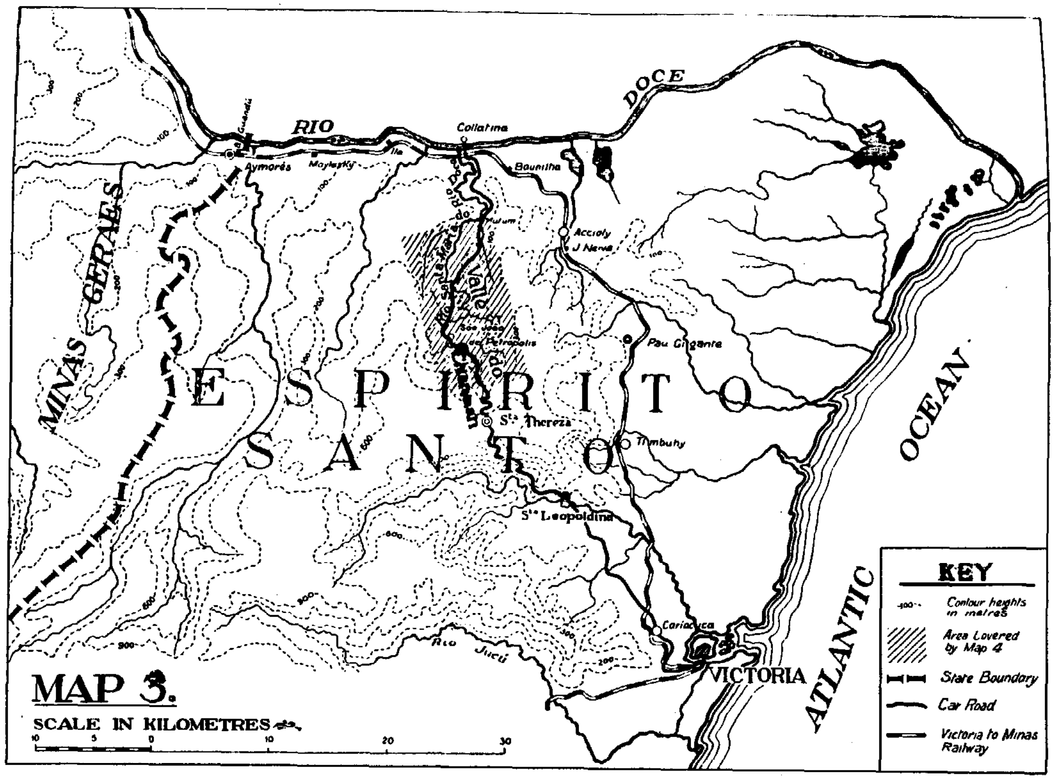
This report is devoted to a strictly rural epidemic of yellow fever, occurring in the Valle do Chanaan, Espirito Santo, Brazil, from January to April, 1932, in which *Aëdes aegypti* can be definitely ruled out as the vector. The results of epidemiological and entomological surveys of the Valle do Chanaan are herewith presented.

Description of the infected area.

The Valle do Chanaan is a delightful and picturesque valley or group of valleys lying between the town of Santa Thereza and the Rio Doce, in the central part of the State of Espirito Santo. This state, one of the smallest in Brazil, has an area of about 45,000 square kilometers and an estimated population of only half a million. It lies almost entirely between the eighteenth and twenty-first degrees of south latitude, on the Atlantic coast. Its location with regard to Rio de Janeiro and Bahia, both of which were formerly important distributing points for yellow fever, is shown on map 1. The bulk of the population is concentrated in the southern half of the state (map 2). The capital and principal seaport, Victoria, has a population of 40,000, and is a port of call for foreign ships from many nations engaged in the coffee trade, as well as for the vessels of several national lines engaged in coast-wise freight and passenger service. The Leopoldina Railway links Victoria with Rio de Janeiro, the Federal Capital, which may be reached in twenty hours (map 1). A second railway line, the Victoria and Minas Geraes, runs north and northwest from Victoria, striking the Rio Doce at Collatina and following the river for some distance into the State of Minas Geraes. The surface of the State of Espirito Santo is very irregular, and some sections are mountainous, although no really high altitudes occur within the state.


MAP 1.
SCALE IN KILOMETRES
State Boundaries.
Rio to Victoria Railway.
Victoria to Minas Railway.
S. SALVADOR
Barra do Rio de Contas
Ilhéos
Belmonte
Alcobaça
Caravellas
BAHIA
MINAS GERAES
ESPIRITO SANTO
RIO DE JANEIRO
SÃO PAULO
BELLO HORISONTE
Cach Escura
Santa Cruz
VICTORIA
São João da Barra
Macahé
Cabo Frio
RIO DE JANEIRO
ATLANTIC OCEAN
42°
12°
16°
20°
24°


MAP 2.
POPULATION MAP
STATE OF ESPIRITO SANTO
BRAZIL
(BASED ON 1920 CENSUS STATISTICS)
●-Represents 2000 inhabitants.
ATLANTIC OCEAN


RIO DOCE
Collatina
Aymorés
Bauniha
Acciolý
J Neiva
Pau Gigante
ESPIRITO SANTO
MINAS GERAES
St. Thereza
Timbuhy
Sta Leopoldina
Rio Jucu
Cariacica
VICTORIA
ATLANTIC OCEAN
KEY
Contour heights in metres
Area Covered by Map 4
State Boundary
Car Road
Victoria to Minas Railway
MAP 3.
SCALE IN KILOMETRES
10 5 0 10 20 30

The Valle do Chanaan (map 3) is formed by the Rio Santa Maria do Rio Doce and its tributaries, where a mountain range falls away from Santa Thereza toward the Rio Doce. The most striking characteristics of this area, immortalized for future generations of Brazilians by Graca Aranha in the romance, "Chanaan," are the narrowness of its valleys and the steepness of its slopes. The automobile highway connecting Victoria and Collatina climbs through Santa Thereza to strike the head of the valley at an altitude of over seven hundred meters. From this vantage point, the Valle do Chanaan is indeed striking, presenting a fairyland picture of steep green hillsides, and narrow well-watered valleys with homes scattered at short distances along the streams. The highway descends rapidly into the valley and traverses it at an average altitude of less than two hundred meters above sea level. The numerous streams of the valley, although they usually descend over rocky beds, are occasionally flanked by small swamps of the type so often found in mountainous districts.

The official census of 1920 showed a population of 18,298 in the Municipio of Santa Thereza, which has a total area of approximately 793 square kilometers. A consideration of the resultant coefficient of twenty-three inhabitants per square kilometer does not convey an adequate idea of the density and distribution of the population in the Valle do Chanaan. This valley is a heavily populated rural area having no villages or towns of importance. The land is divided into relatively small holdings; and since practically all homes are built along the streams, the distance from any house to its nearest neighbor is seldom more than a few hundred meters. The area was colonized during the latter decades of the past century by Italian and German immigrants, and these stocks still predominate. The principal export crop of the region is coffee, in the care and gathering of which all members of the family generally participate. The only points of concentration of population in the valley are São João de Petropolis and Patrimonio de Santo Antonio, with about fifty houses each. The absence of towns has given rise to a system of names for different parts of the valley which is very difficult for the newcomer and apparently somewhat uncertain for the native. A few places are referred to by the name of the patron saint of the local church, but the great majority take the name of nearby streams. At times a rather vague distinction is made between the mouth of the stream, the stream itself, and its headwaters. Considering the mountainous character of the country this system often results in places hours apart by horseback travel being referred to by the same name. Off the main highway, travel is slow and difficult and is properly indulged in only on foot or on horseback.

History of yellow fever in Espirito Santo.

Considering its strategic position between Rio de Janeiro and Bahia, Espirito Santo could not but have a long and funereal yellow fever history during the period from 1849 to 1908, when the disease was endemic both to the north and to the south. With the development of European colonization, Victoria became notorious as a yellow fever center, and at least one foreign government took measures during the last decade of the nineteenth century to prevent emigration of its nationals to Espirito Santo. With the disappearance of yellow fever from southern Brazil following the early antimosquito campaigns, Victoria reported no cases of the disease for several years, but finally it suffered from a sharp outbreak during 1917 and 1918. Two suspected cases occurred in Santa Leopoldina in 1925, but these were not confirmed by the official investigation; and no yellow fever was reported for the entire state during thirteen years (1919–31). With the reappearance of yellow fever in 1928 in the Federal Capital, only twenty hours from Victoria either by boat or by rail, the State Health Department of Espirito Santo organized control measures and maintained them until April, 1931, when the administration of the antimosquito service passed to the Cooperative Yellow Fever Service maintained by the National Department of Health and The Rockefeller Foundation. In December, 1930, in preliminary discussions with one of us (FLS) of the possible continued endemicity of yellow fever in Espirito Santo as an explanation of the failure of this state to produce visible autochthonous cases of the disease during the years 1928, 1929, 1930, and 1931, when it is known to have been present in the neighboring state of Rio de Janeiro, Dr. Alvaro Mello, Director of the State Health Service, mentioned the Municipio of Santa Thereza, of which the Valle do Chanaan is a part, as a suspected area. As a result of this discussion the attention of the Yellow Fever Service in Victoria was called to this county, but many months passed during which no suspicious cases were recorded. In January, 1932, Dr. A. M. Walcott visited the State of Espirito Santo to organize the service for the routine collection of liver specimens from patients with fatal fever cases of short duration. In discussing the distribution of this special service, the object of which is the discovery of otherwise unknown foci of yellow fever, Dr. Mello again mentioned Santa Thereza as an area requiring investigation. Dr. Walcott visited the town of Santa Thereza, lying more than six hundred meters above sea level, found no *Aëdes aegypti*, and decided against installing a liver-collection service. Driving to the top of the pass beyond Santa Thereza he looked down upon the beauties of the

Valle do Chanaan, hundreds of meters below, but, like Moses of old, who gazed upon but did not enter the Valley of Canaan, Walcott turned back. Santa Thereza and the Valle do Chanaan were logically struck from the list of places needing further investigation. Subsequent events were to prove that yellow fever had been present in the Valle do Chanaan when this decision was made.

Discovery of of yellow fever in Valle do Chanaan.

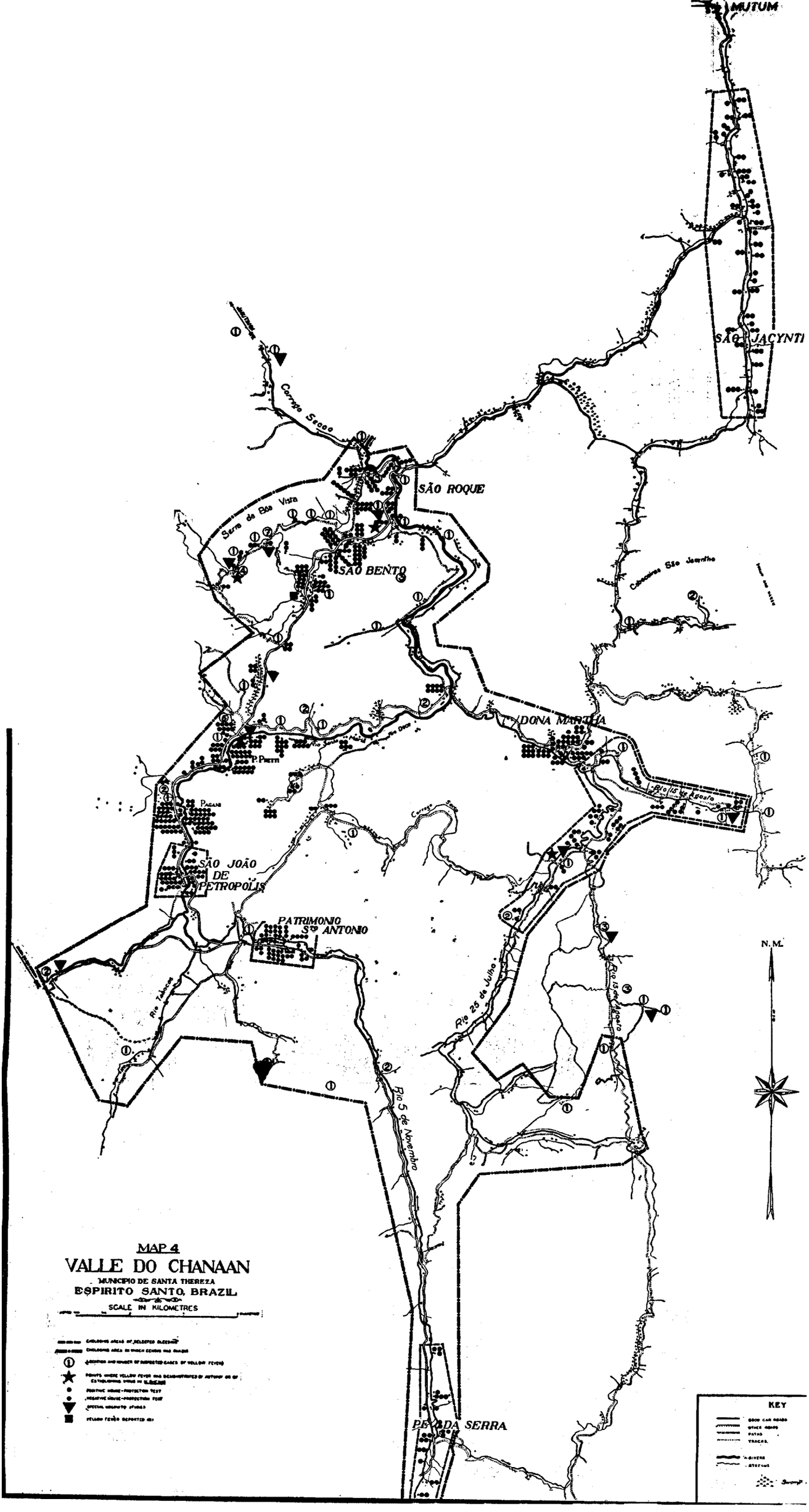
On March 3, 1932, Dr. Arnaldo de Andrade, representative of the State Health Service for Santa Thereza, reported a suspected case of yellow fever. This case proved fatal and an autopsy was performed by Dr. Andrade and Dr. Americo Oliveira. Examination of liver tissues by Dr. Amadeu Fialho and Dr. N. C. Davis resulted in a confirmation of the clinical diagnosis.

Epidemiological investigation.

Field investigations, which were begun immediately, resulted in a second positive autopsy a few days after the first and in a third positive autopsy on the 26th of March. The presence of yellow fever in the Valle do Chanaan was further demonstrated by the fact that Dr. Davis was able to establish the virus in *Macacus rhesus* at the Bahia Yellow Fever Laboratory, by the injection of serum from a patient in this area from whom a blood specimen was taken on April 3. The places where yellow fever was shown, by autopsy and by animal inoculation, to exist are indicated on map 4 and are seen to be widely separated. Actually, because of the difficulties of travel, these infected points are much more isolated than they appear to be.

Dr. Alvaro Mello, Director of the Health Department, installed hospital facilities in São João de Petropolis. Here Dr. Arnaldo de Andrade attempted to concentrate for study all acute febrile cases occurring in the valley. The authors are deeply indebted to these colleagues for their whole-hearted cooperation, without which this study would have been most difficult. It soon became apparent that cases of typhoid and malaria were occurring in the region, as well as cases of yellow fever, which were few in number and widely scattered. Finally, on April 21, the São João Hospital was turned over to the Yellow Fever Service as a laboratory for carrying out transmission experiments.

Our earliest definite clue to the presence of yellow fever in the Valle do Chanaan was the report of three cases, one fatal, which had occurred in the 13 de Agosto Valley, with onset on January 19, 1932. The description of these cases given by the attending physician and by the surviving patients agrees with the classical picture of yellow fever.


MUTUM
SÃO JACYNTI
Corrego Secco
SÃO ROQUE
Serra de Boa Vista
SÃO BENTO
Corrego São Jasmino
DONA MARTHA
P. Pagani
P. Pietri
Rio 15 de Agosto
SÃO JOÃO DE PETROPOLIS
PATRIMONIO Sº ANTONIO
Rio Tabocas
Rio 25 de Julho
Rio 5 de Novembro
PEDRA DA SERRA
N.M.
MAP 4
VALLE DO CHANAAN
MUNICIPIO DE SANTA THEREZA
ESPIRITO SANTO, BRAZIL
SCALE IN KILOMETRES
KEY

However, they could scarcely have been the original cases, since all the patients fell ill on the same day. Further evidence that these were not the first cases in the area is furnished by the second known fatal case, which occurred on February 1 in the 15 de Agosto Valley, at some distance from the first fatal case and without any apparent contact with this focus. A less convincing case of eight days' duration in an employee twenty-two years of age, who had had no medical attention, was reported by a family in the 5 de Novembro Valley as dating back to the beginning of the summer, in November, 1931. In the same month this family lost a two-year old child, but they were unable to supply satisfactory details of its illness.

Information was collected regarding a total of eighty-three suspected cases with nine fatalities which occurred between January 15 and April 15, and which were scattered over an area of some fifty square kilometers. The epidemic was apparently on the decline when it was discovered, and only twenty-one cases, with three deaths, were observed after the appearance of the first suspected case. This apparent low mortality of yellow fever in a population predominantly white and largely composed of European stocks recently established in this area, emphasizes the fact that other factors than race are important in determining susceptibility to yellow fever. It is believed that the number of deaths reported is close to the number actually caused by yellow fever, but it is hard to estimate the number of infections, since difficult travel conditions made repeated visits to suspected cases impossible. The distribution of cases believed to have been yellow fever is shown in table 1 and map 4; and the time of onset, by weeks, is shown in table 2. In considering this information, it must be remembered that conclusions regarding happenings previous to the first week in March are based largely on information furnished by unskilled observers. With cases of typhoid and malaria both appearing in the infected area, it is probable that there are several errors in the data presented.

During the field investigation, various persons stated that this was not the first time that "black typhus" had been present in the Valle do Chanaan, but that it appeared at intervals of several years, killing a few persons in the valley and then disappearing. The only definite reference to the previous existence of yellow fever in this region was made by a widow who said that her husband had died of the disease in 1911. One of her two children, who was reported to have had the disease at the same time, was found by the protection test to be immune to yellow fever.

TABLE 1.

Suspected cases of yellow fever occurring in Valle do Chanaan, January to April, 1932.

Date of onset	Case	Residence	House number	Record number	Outcome	Confirmed by			
						Autopsy	Animal inoculation	Mouse protection	Clinical observation
Jan. 18	AM	13 de Agosto	—	—	Fatal	—	—	—	+
20	-M	13 de Agosto	—	—	Recovery	—	—	—	—
20	-M	13 de Agosto	—	—	Recovery	—	—	—	—
Feb. 1	AS	15 de Agosto	—	—	Fatal	—	—	—	+
3	DMO	São Jacyntho	—	—	Fatal	—	—	—	—
7	JL	Serra Bôa Vista	92	727	Recovery	—	—	+	—
8	MF	São Roque	70	509	Recovery	—	—	+	—
9	HV	Serra Bôa Vista	90	—	Fatal	—	—	—	—
10	JF	Serra Bôa Vista	92	727	Recovery	—	—	—	—
10	J-	São Roque	70	—	Recovery	—	—	—	—
13	DV	Serra Bôa Vista	90	—	Fatal	—	—	—	—
15	EWB	25 de Julho	199	—	Recovery	—	—	—	—
16	MSB	25 de Julho	199	3258	Recovery	—	—	—	—
20	RC	13 de Agosto	—	—	Recovery	—	—	—	—
20	AC	13 de Agosto	—	—	Recovery	—	—	—	—
20	LC	13 de Agosto	—	—	Recovery	—	—	—	—
23	AR	13 de Agosto	—	—	Recovery	—	—	—	—
24	EV	Serra Bôa Vista	90	711	Recovery	—	—	—	—
24	GV	Serra Bôa Vista	90	394	Recovery	—	—	+	—
24	AV	Serra Bôa Vista	90	—	Recovery	—	—	+	—
25	AV	Serra Bôa Vista	90	—	Fatal	—	—	—	—

TABLE 1 (*Continued*).

Date of onset	Case	Residence	House number	Record number	Outcome	Confirmed by			
						Autopsy	Animal inoculation	Mouse protection	Clinical observation
26	–V	Serra Bôa Vista	90	—	Recovery	—	—	—	—
26	JF	Serra Bôa Vista	88	691	Recovery	—	—	—	—
26	RP	Santa Maria	315	2469	Recovery	—	—	—	—
27	OP	Santa Maria	315	2468	Recovery	—	—	—	—
27	JV	Serra Bôa Vista	428	3216	Recovery	—	—	—	—
27	PR	13 de Agosto	636	2165	Recovery	—	—	—	—
28	HC	15 de Agosto	356	2764	Recovery	—	—	—	—
29	JM*	Santa Maria	18	—	Fatal	+	—	—	+
Date uncertain but previous to March 6	CC	Santa Maria	74	520	Recovery	—	—	—	—
	MC	Santa Maria	74	522	Recovery	—	—	—	—
	AC	Santa Maria	74	525	Recovery	—	—	—	—
	DC	Santa Maria	74	530	Recovery	—	—	+	—
	EF	Santa Maria	74	532	Recovery	—	—	+	—
	AL	Serra Bôa Vista	91	724	Recovery	—	—	+	—
	PB	Santa Maria	394	3014	Recovery	—	—	—	—
	FAR	São Roque	—	—	Recovery	—	—	—	—
	SG	Santa Maria	390	3001	Recovery	—	—	—	—
	JNS	São Roque	409	3112	Recovery	—	—	—	—
	GR	13 de Agosto†	—	—	Recovery	—	—	—	—
	MC	São Roque	70	505	Recovery	—	—	+	—
	OR	13 de Agosto	—	—	Recovery	—	—	—	—
	FC	Ribeirão S. José†	—	—	Recovery	—	—	—	—
	TR	Ribeirão S. José†	—	—	Recovery	—	—	—	—
	CC	Ribeirão S. José†	—	—	Recovery	—	—	—	—

* First case reported and confirmed.

† Not shown on map 4.

TABLE 1 (*Continued*).

Date of onset	Case	Residence	House number	Record number	Outcome	Confirmed by			
						Autopsy	Animal inoculation	Mouse protection	Clinical observation
March 1	LB	São Roque	429	—	Recovery	—	—	—	—
1	JT	São Jacyntho	—	—	Recovery	—	—	—	—
1	AP	Santa Maria	307	2411	Recovery	—	—	—	—
1	JM	Santa Julia	—	—	Recovery	—	—	—	Hospitalized
2	SR	Picadão	36	39	Recovery	—	—	+	+
2	ZT	São Jacyntho	—	—	Recovery	—	—	—	—
3	AB	Rio Perdido	518	51	Recovery	—	—	+	+
3	GB	Rio Perdido	518	52	Recovery	—	—	+	+
3	GO	Santa Maria	303	2383	Recovery	—	—	—	Hospitalized
3	CW	Picadão	8	81	Recovery	—	—	+	+
3	TT	Santa Maria	397	3034	Recovery	—	—	+	—
3	FT	Picadão	416	315	Recovery	—	—	—	—
3	PL	Serra Bôa Vista	92	—	Fatal	+	—	—	—
3	AT	Santa Maria	401	3071	Recovery	—	—	—	Hospitalized
4	FZ	Santa Maria	85	607	Recovery	—	—	—	+
4	TT	Santa Maria	401	3070	Recovery	—	—	—	Hospitalized
5	OZ	Santa Maria	85	608	Recovery	—	—	—	+
7	AF	25 de Julho	635	2154	Recovery	—	—	—	+
8	HG	Serra Bôa Vista	89	705	Recovery	—	—	—	Hospitalized

TABLE 1 (*Continued*).

Date of onset	Case	Residence	House number	Record number	Outcome	Confirmed by			
						Autopsy	Animal inoculation	Mouse protection	Clinical observation
March 9	LL	Serra Bôa Vista	92	725	Recovery	−	−	−	+
10	–D	5 de Novembro	−	−	Recovery	−	−	−	−
10	–D	5 de Novembro	−	−	Recovery	−	−	−	−
18	JT	Santa Maria	401	3069	Recovery	−	−	−	Hospitalized
20	PP	Jacutinga	−	−	Recovery	−	−	−	Hospitalized
21	JS	5 de Novembro	−	−	Recovery	−	−	−	+
22	WN	25 de Julho	196	−	Fatal	+	−	−	+
26	JAS	15 de Agosto	−	−	Recovery	−	−	−	+
27	MPS	Corrego Secco	600	1943	Recovery	−	−	−	Hospitalized
April 1	JG	São Roque	66	481	Recovery	−	−	+	Hospitalized
1	AV	H. Rio Tabocas	547	1416	Recovery	−	−	−	+
2	JF	São Roque	67	490	Recovery	−	+	−	Hospitalized
3	AG	Patrimonio Sto. Antonio	616	2038	Recovery	−	−	−	+
4	CB	15 de Agosto	189	2550	Recovery	−	−	−	+
4	FD	Rio Tabocas	530	1227	Recovery	−	−	−	Hospitalized
4	BM	Santa Maria	305	2398	Recovery	−	−	−	Hospitalized
5	MF	Santa Maria	305	−	Recovery	−	−	−	Hospitalized
13	APS	Picadão	82	581	Recovery	−	−	+	Hospitalized
14	OR	13 de Agosto	−	−	Recovery	−	−	−	+

TABLE 2.

Date of onset of suspected and confirmed cases of yellow fever in the Valle do Chanaan, 1932.

Date	Estimated week of epidemic	Known distribution	
		Cases	Deaths
Jan. 18–24	1	3	1
25–31	2	0	0
Feb. 1– 7	3	3	1
8–14	4	5	2
15–21	5	5	0
22–28	6	12	1
29–Mar. 6	7	18	2
Mar. 7–13	8	5	0
14–20	9	1	0
21–27	10	4	1
28–Apr. 3	11	4	0
Apr. 4–10	12	4	0
11–17	13	2	0
18–24	14	0	0
Subtotal	–	66	8
Undated cases previous to March 6	–	17	1
Total	–	83	9

Beginning May 1, fifteen days after the appearance of the last observed case of suspected yellow fever, and continuing through the month of June, a house-to-house census was carried out, in various parts of the Valle do Chanaan (map 4). During this census, 582 blood specimens were secured from among 3262 persons residing in 496 houses situated in what may be termed the suspected area. These specimens were distributed as follows:

1. Four hundred and twelve routine specimens from among 618 persons living in the first eighty-seven houses visited in the rural areas north and east of São João de Petropolis, listed under "Routine Bleedings" in table 3.

2. One hundred and sixty-eight specimens from São João de Petropolis, Patrimonio de Santo Antonio, Pe da Serra, and an area lying south and east of Dona Marta, and including sections of 15 de Agosto, 13 de Agosto, and 25 de Julho, listed under "Selective Bleedings" in table 4. The samples in this group were limited to not more than two from any family, and were from persons native to the Valle do Chanaan.

TABLE 3.

Result of mouse-protection test by age of donor of serum and place of residence in Valle do Chanaan.

Age group (years)	Routine bleeding								*Total*		
	Pagani		*P. Pretti and Sta. Maria*		*S. Bôa Vista São Bento São Roque*		*Dona Marta*				
	Number of sera tested	Number of sera protecting	Number of sera tested	Number of sera protecting	Number of sera tested	Number of sera protecting	Number of sera tested	Number of sera protecting	Number of sera tested	Number of sera protecting	Per cent of sera protecting
0– 9	17	1	13	0	28	1	4	0	62	2	3.2±1.5
10–19	16	1	43	0	45	9	17	0	121	10	8.3±1.7
20–29	19	1	32	4	49	10	15	2	115	17	14.8±2.2
30–39	12	4	16	3	32	10	9	0	69	17	24.6±3.5
40 and over	10	3	16	7	9	1	10	5	45	16	35.6±4.8
Total	74	10	120	14	163	31	55	7	412	62	15.0±1.2
Per cent of sera protecting	13.5±2.7		11.7±2.0		19.0±2.1		12.7±3.0		15.0±1.2		

TABLE 4.

Results of mouse-protection test by age of donor of serum and place of residence in Valle do Chanaan.

Age group (years)	Selective bleeding (See page 568)										Total		
	Pé da Serra		*Patrimonio de Santo Antonio*		*São João de Petropolis*		*São Jacyntho*		*13 de Agosto 15 de Agosto 25 de Julho*				
	Number of sera tested	Number of sera protecting	Number of sera tested	Number of sera protecting	Number of sera tested	Number of sera protecting	Number of sera tested	Number of sera protecting	Number of sera tested	Number of sera protecting	Number of sera tested	Number of sera protecting	Per cent of sera protecting
0– 9.....	9	0	15	0	13	0	23	3	15	0	75	3	4.0±1.5
10–19.....	6	0	14	0	12	0	21	0	21	1	74	1	1.4±0.9
20–29.....	7	0	9	0	7	0	15	1	8	1	46	2	4.3±2.0
30–39.....	4	0	6	1	5	1	7	0	7	1	29	3	10.3±3.8
40 and over..	1	0	2	0	3	3	2	1	4	1	12	5	14.2±6.8
Total.....	27	0	46	1	40	4	68	5	55	4	236	14	5.9±1.0
Per cent sera protecting..	0		2.2±1.5		10.0±3.2		7.4±2.1		7.3±2.4		5.9±1.0		

As a control on specimens collected from the suspected area, sixty-eight samples, representing forty-one families, were secured from persons born in the zone called São Jacyntho, lying just outside the known infected zone and close to Mutum, the nearest known source of *Aëdes aegypti*. The results of tests on these specimens are not significantly different from those in the known infected area, 13 de Agosto, 15 de Agosto, and 25 de Julho, and they are included in table 4 under "Selective Bleedings," together with results for specimens selected in the same manner in the infected area.

The occurrence of a fatal autopsied case in a man seventy-two years of age, a resident in the Valle do Chanaan for fifty-five years, suggested that this area was not highly immunized. The results of mouse-protection * tests in the valley showed a surprisingly low percentage of immune persons, considering the wide geographical distribution of immunity and the fact that the disease apparently disappeared spontaneously. This situation might well have been due to transmission by some insect vector showing high efficiency in the laboratory but low

TABLE 5.

History of recent suspicious illness among persons with positive mouse-protection tests who had probably acquired immunity to yellow fever in the Valle do Chanaan.

Age group (years)	Number of immunes	Persons giving history of recent suspicious illness	Total number of immunes	Total number of persons giving history of recent suspicious illness
0- 9	3	2	25	12
10-19	10	3		
20-29	12	7		
30-39	15	3	28	3
40 and over	13	0		
Total	53	15	53	15

efficiency in nature because of non-domestic habits, and consequently less contact with the human host than *Aëdes aegypti* has. The very small percentage of immune persons found in the age group 10 years and under and the absence of cases in the small hamlets of São João de Petropolis and Patrimonio de Santo Antonio, are both possible of explanation on the basis of field transmission. On the other hand, the age distribution of positives might be interpreted as a result of repeated

* All protection tests reported in the present study were made by Dr. Henrique Penna at the Yellow Fever Laboratory of The Rockefeller Foundation, in Bahia.

visits of the disease with a small number of cases during each invasion. Such repeated invasions with few cases would, in the course of time, build up higher percentages of immunity in the older age groups. This fact seems to be confirmed by the study by age groups of the history of suspected illness among positives who probably acquired their immunity in the Valle do Chanaan (table 5). This study shows a much higher percentage of young than of old positives, among those having a history of recent attack.

Entomological investigations.

Inspectors sent to the area immediately upon the report that yellow fever existed there failed to find *Aëdes aegypti* breeding in the Valle do Chanaan * or in the town of Santa Thereza. Detailed studies carried out during many weeks by highly trained workers failed to reveal larvae or adults of this mosquito. Nearby towns showed high indices of *A. aegypti* breeding, but no recognized cases of yellow fever. The comparative findings regarding the prevalence of *A. aegypti* on first inspection, for different points in the Valle do Chanaan and for nearby apparently uninfected towns, are given in table 6. This table, in the absence of positive autopsies and transfer of virus to *M. rhesus*, must have resulted in the denial of the existence of yellow fever in the Valle do Chanaan.

The absence of *Aëdes aegypti* from the Valle do Chanaan probably depends on several factors, among which may be mentioned: (1) the small number of water containers inside the houses, as a result of the location of practically all homes on the banks of small mountain streams or rivers, which obviates entirely the necessity of storing water for domestic purposes; (2) the almost complete absence of artificial water containers outside the homes; (3) the relatively brusque changes in meteorological conditions, due probably not so much to the altitude of the valley as to its formation and its relationship to the surrounding mountains. Unfortunately, adequate meteorological data are not available. The limited observations made during the present study at São João de Petropolis were begun only after the epidemic was over. That weather conditions can change rapidly here is well shown by the data for June 4: temperature, maximum 40° C., minimum 15° C.; relative humidity, maximum 94 per cent, and minimum 42 per cent.

* Among the first foci collected in São João de Petropolis, one contained larvae classified as *Aëdes aegypti* by Dr. A. Lutz. Most careful search failed to reveal other foci of the same mosquito. It is not known whether this focus represented an accidental one brought in from nearby points where *A. aegypti* is common, or was really from some other place and forwarded in a mislabelled container.

That the area is not suitable for *Aëdes aegypti* breeding is emphasized by the low percentage of houses (3.2 ± 1.5) with larvae of this species in Mutum, the nearest point where larvae were found.

TABLE 6.

Comparative results of first inspection for Aëdes aegypti *breeding in the Valle do Chanaan and nearby points, March, 1932.*

Yellow fever present				Yellow fever not found			
Valle do Chanaan				Localities outside Valle do Chanaan			
District	Houses		House index	Town	Houses		House index
	Examined	With *Aëdes aegypti*			Examined	With *Aëdes aegypti*	
Patrimonio Sto. Antonio	54	0	0	Santa Thereza*	153	0	0
S. João de Petropolis	52	0	0	Santa Leopoldina	322	26	8.1±1.0
15 de Agosto	55	0	0	Timbuhy	75	11	14.7±2.8
25 de Julho	64	0	0	Accioly	60	9	15.0±3.1
Bôa Vista, Santa Maria, São Roque	96	0	0	Collatina	534	101	18.9±1.1
				Maylasky	44	16	36.4±4.9
				Cariacica	258	25	9.7±1.2
				Aymorés	100	31	31.0±3.1
				Baixo Guandú	65	19	29.2±3.8
				Mutum	65	2	3.1±1.4
Many examinations were made later in these and other districts of the Valle do Chanaan with uniformly negative results for *Aëdes aegypti*.				Itá	70	17	24.3±3.5
				Baunilha	39	3	7.7±2.9
				João Neiva	53	17	32.1±4.3
				Pau Gigante	60	8	13.3±3.0

* More than 600 meters above sea-level.

Early attempts to determine the common mosquitoes of this area by the capture of adults inside the houses at various hours of the day and night were almost fruitless. Occasionally one to four mosquitoes might be found in a house, but at no time during the present studies were appreciable numbers of any species of mosquito captured inside houses. The first reaction to this failure to find mosquitoes in the houses was to hypothecate transmission by some blood-sucking parasite other than the mosquito. Search of houses for such insects, however, failed to produce suggestive results, except that the possibility of the implication of *Phlebotomi* was presented. Triatoma, bedbugs, fleas, and ticks were not commonly found during the months of

this study.* Accidental transmission through parasites of domestic animals seems impossible, since the number of such animals is small.

Only when adult mosquito captures were undertaken with animal bait outside of, but close to, houses, did the wealth of mosquito life become apparent. The number of mosquitoes of different species found outside the houses proved to be in marked contrast to the scarcity of mosquito life in the houses. During March, April, and May extensive adult captures at all hours of the day and night, supplemented by the collection of larvae and the identification of foci, were made at eleven points where local transmission of yellow fever had almost certainly occurred. As a control on this work, less exhaustive investigations were made at twenty-one other houses, most of which had produced suspected cases of yellow fever. These investigations failed to produce any adults or larvae of *Aëdes aegypti*. The following species of mosquitoes and *Phlebotomi* † were captured or identified from larvae secured in the Valle do Chanaan:

Aëdes scapularis, fluviatilis, serratus, terrens, fulvus, jacobinae, leucocoelenus, rhyacophilus ‡

Mansonia titillans, juxtamansonia, chrysonotum, albicosta, fasciolata, indubitans

Microculex imitator, pleuristriatus, and unidentified species

Culex corniger, nigripalpus, conservator, surinamensis, coronator (*Mochlostyrax*) various species, (*Melanoconion*) various species, and other unidentified species

Anopheles tarsimaculatus, argyritarsis, albitarsis, bachmanni, darlingi, brasiliensis, fluminensis, intermedius, maculipes, minor, (*Kertezia*) *cruzii,* (*Chagasia*) *fajardoi*

Psorophora cingulata, ciliata, ciliipes, ferox, lutzii

* Davis (1) has recently reported extensive transmission experiments with *Argas persicus* (Oken), *Amblyomma cajennense* (Fabricius), *Rhipicephalus sanguineus* (Latreille) and *Boophilus microplus* (Canestrini) with negative results by bite and by injection of eggs of infected females. The virus was shown to survive for periods of six to twenty-eight days in individuals of the various species studied.

Aragão (2) subsequently reported transmission with *Amblyomma cajennense* (Fabricius) by bite and (personal communication) by emulsion of eggs from infected females.

(1) Davis, N. C. *The Survival of Yellow Fever Virus in Ticks.* (In Press.)

(2) Aragão, H. *Transmissão da Febre Amarella por Carrapatos.* Brasil-Medico XLVII, No. 11, March 18, 1933, p. 185–186.

† The authors are indebted to Dr. A. Lutz, Dr. A. da Costa Lima, and Dr. N. C. Davis for valuable assistance in classifying insects from the Valle do Chanaan.

‡ *Aëdes* (*Ochlerotatus*) *rhyacophilus,* sp.n., first discovered in the present survey, is to be described by Prof. A. da Costa Lima.

Wyeomyia oblita, bromeliarum, tripartita, pallidoventer, unidentified species
Dendromyia personata, melanoides, mystes, unidentified species
Megarhinus violaceus, fluminensis
Uranotaenia geometrica, lowii, pulcherrima
Joblotia digitata
Haemagogus equinus
Limatus durhami
Isostomyia, unidentified species
Miamyia, unidentified species
Sabethoides, unidentified species
Phlebotomus intermedius, fischerii, migonei

Although the fauna collected was extensive, additional species should be encountered in surveys conducted earlier in the summer, when the temperature is higher and the rainfall heavier.

In view of the ~~fact that much~~ work already been done on transmission with various species of mosquitoes in the laboratory (table 7), the following species were chosen from among those captured, for further consideration as possible vectors in the 1932 outbreak in the Valle do Chanaan: *Aëdes fluviatilis, Aëdes scapularis, Aëdes serratus, Aëdes terrens, Mansonia* (all species), *Psorophora* (all species), and *Phlebotomus* (all species). *Phlebotomus* was included in the list because no work had been reported on this insect. The comparative incidence of adults and of larvae of the above-mentioned species, found at eleven points where there is reasonable certainty that local transmission of yellow fever occurred (table 8), suggests that *Aëdes scapularis* may well have been the vector during the outbreak studied. *Aëdes scapularis* was found at all eleven places, *Aëdes fluviatilis* at five, *Aëdes serratus* and *Aëdes terrens* at one place each, *Mansonia* (all species) at eight places, *Phlebotomus* (all species) at six places, and *Psorophora* (all species) at three places. Adult captures with animal bait at these eleven points included 224 *Aëdes scapularis,* 12 *Aëdes fluviatilis,* 652 *Mansonia spp.,* 1486 *Phlebotomi spp.,* and 12 *Psorophora spp.* However, uncommonly swampy conditions existed at one place of capture (no. 11, APS), and repeated catches were made here to secure *Phlebotomi* for transmission experiments. Leaving out the figures for no. 11, the totals for adult captures become: *Aëdes scapularis* 208, *Mansonia* 176, and *Phlebotomi* 210. *Aëdes scapularis* proved to be a ready and vicious biter.*

* Preferred feeding periods observed were from 5:00 to 10:00 P.M. and 4:00 to 7:00 A.M.

TABLE 7.

Summary of reported experiments on transmission of yellow fever virus by mosquitoes.

Species	Source of mosquitoes used	Results of biting tests		Results of inoculation tests		Reported by
		Positive or negative	Least number of days positive	Positive or negative	Greatest number of days positive	
Aëdes (*Stegomyia*) *aegypti* (Linn.)	Cuba	+	12 to 4*	−	−	Reed, et al, 1901 (11)
Aëdes (*Stegomyia*) *africanus* (Theob.)	West Africa	+	12	−	−	Philip, 1929 (2)
Aëdes (*Stegomyia*) *albopictus* (Skuse)	Java	+	?	−	−	Dinger et al, 1929 (12)
Aëdes (*Aedimorphus*) *apicoannulatus* (Edw.) (renamed *stokesi*, Edw.)	West Africa	+	19	−	−	Bauer, 1928 (1)
Aëdes (*Stegomyia*) *apicoargenteus* (Theob.)	West Africa	0	−	0	−	Bauer, 1928 (1) and also Evans, 1929 (13)
Aëdes (*Taeniorhynchus*) *fluviatilis* (Lutz)	Brazil	+	16	−	−	Davis and Shannon, 1931 (7)
Aëdes (*Howardina*) *fulvithorax* (Lutz)	Brazil	0	−	0	−	Davis and Shannon, 1931 (8)
Aëdes (*Aedimorphus*) *irritans* (Theob.)	West Africa	0	−	+	51	Kerr, 1932 (16)
Aëdes (*Stegomyia*) *luteocephalus* (Newst.)	West Africa	+	15	−	−	Bauer, 1928 (1)
Aëdes (*Aedimorphus*) *nigricephalus* (Theob.)	West Africa	0	−	+	?	Philip, 1930 (4)
Aëdes (*Banksinella*) *punctocostalis* (Theob.)	West Africa	0	−	+	?	Philip, 1930 (4)
Aëdes (*Ochlerotatus*) *scapularis* (Rondani)	Brazil	+	13	−	−	Davis and Shannon, 1929 (6)
Aëdes (*Stegomyia*) *scutellaris* (same as *albopictus*)	Far East	+	?	−	−	De Vogel, 1930 (15)
Aëdes (*Ochlerotatus*) *serratus* (Theob.)	Brazil	0	−	+	31	Davis and Shannon, 1929 (6)
Aëdes (*Stegomyia*) *simpsoni* (Theob.)	West Africa	+	19	−	−	Philip, 1929 (2)
Aëdes (*Aedimorphus*) *stokesi* (Edw.) (reported to be same as *apicoannulatus*, Edw.)	West Africa	+	19	−	−	Bauer, 1928 (1)

TABLE 7 (*Continued*).

Species	Source of mosquitoes used	Results of biting tests		Results of inoculation tests		Reported by
		Positive or negative	Least number of days positive	Positive or negative	Greatest number of days positive	
Aëdes (*Taeniorhynchus*) *taeniorhynchus* (Wiedemann)	Brazil	+	19	—	—	Davis and Shannon, 1931 (7)
Aëdes (*Finlaya*) *terrens* (Walker)	Brazil	0	—	+	14	Davis and Shannon, 1931 (8)
Aëdes (*Stegomyia*) *vittatus* (Bigot)	West Africa	+	11	—	—	Philip, 1929 (2)
Anopheles (*Nyssorhynchus*) *albitarsis* (Lynch Arribalzaga)	Brazil	0	—	0	—	Davis and Shannon, 1931 (8)
Anopheles (*Myzomyia*) *gambiae* (Giles)	West Africa	0	—	0	—	Philip, 1930 (3)
Anopheles (*Nyssorhynchus*) *tarsimaculatus* (Goeldi)	Brazil	0	—	0	—	Davis and Shannon, 1931 (8)
Culex (*Culex*) *thalassius* (Theob.)	West Africa	+	27	—	—	Kerr, 1932 (16)
Culex (*Culex*) *quinquefasciatus* (Say)†	Brazil	0	—	0?	—	Davis and Shannon, 1929 (6)
		0	—	—	—	Marchoux and Simond, 1906 (14)
		0	—	—	—	Reed et al, 1911 (11)
Eretmopodites chrysogaster (Graham)	West Africa	+	16	—	—	Bauer, 1928 (1)
Joblotia digitata (Rondani)	Brazil	0	—	0	—	Davis and Shannon, 1931 (8)
Limatus durhami (Theob.)	Brazil	0	—	0	—	Davis and Shannon, 1931 (7)

TABLE 7 (*Continued*).

Species	Source of mosquitoes used	Results of biting tests		Results of inoculation tests		Reported by
		Positive or negative	Least number of days positive	Positive or negative	Greatest number of days positive	
Mansonia (*Mansonioides*) *africanus* (Theob.)	Africa	+	16	−	−	Philip, 1930 (3)
Mansonia (*Rhynchotaenia*) *albicosta* (Peryassú)	Brazil	0	−	+	−	Davis and Shannon, 1931 (8)
Mansonia (*Mansonia*) *chrysonotum* (Peryassú)	Brazil	0	−	+	−	Davis and Shannon, 1931 (8)
Mansonia (*Rhynchotaenia*) *fasciolata* (Lynch Arribalzaga)	Brazil	0	−	+	−	Davis and Shannon, 1931 (8)
Mansonia (*Mansonia*) *titillans* (Walker)	Brazil	0	−	+	30	Kumm and Frobisher, 1932 (18)
Mansonia (*Mansonioides*) *uniformis* (Theob.)	West Africa	0	−	++	24	Philip, 1930 (4)
Psorophora (*Psorophora*) *ciliata* (Fabricius) (Early work Marchoux and Simond)	Brazil	0	−	0	−	Marchoux and Simond, 1906 (14)
Psorophora (*Grabhamia*) *cingulata* (Fabricius)	Brazil	not tested	−	+	15	Davis and Shannon, 1931 (8)
Psorophora (*Janthinosoma*) *ferox* (Humboldt)†	Brazil	0	−	+	20	Davis and Shannon, 1931 (8)
Psorophora (*Janthinosoma*) *posticata* (Wied.) (Early work Marchoux and Simond) (same species as *ferox*)	Brazil	0	−	0	−	Marchoux and Simond, 1906 (14)
Wyeomyia (*Pentemyia*) *bromeliarum* (Dyar and Knab)	Brazil	0	−	0	−	Davis and Shannon, 1931 (7)
Wyeomyia (*Wyeomyia*) *oblita* (Theob.)	Brazil	0	−	0	−	Davis and Shannon, 1931 (7)

* Has been shown to vary with temperature. (Davis, 1932 (9).)

† Since the preparation of this table, Dr. Nelson C. Davis has succeeded in transmitting the virus of yellow fever to *Macacus rhesus* by the bites of *Culex* (*Culex*) *quinquefasciatus* (Say) and *Psorophora* (*Janthinosoma*) *ferox* (Humboldt). (Unpublished communication.)

TABLE 8.

Partial summary of entomological findings at eleven places where local transmission of yellow fever probably occurred.

Study place number Map 4	*Mansonia* all species		*Aëdes scapularis*		*Aëdes fluviatilis*		*Aëdes serratus*		*Aëdes terrens*		*Phlebotomi* all species		*Psorophora* all species	
	Adults	Larvae	Adults	Larvae	Adults	Larvae	Adults	Larvae	Adults	Larvae	Adults	Larvae	Adults	Larvae
5	+	+	+	+	0	0	0	+	0	0	+	0	0	0
7	+	0	+	+	+	0	0	0	+	+	+	0	+	0
8	0	0	+	0	0	0	0	0	0	0	+	0	0	0
9	+	0	+	+	+	0	0	0	0	0	0	0	+	+
10	+	0	+	0	+	+	0	0	0	0	+	0	0	0
11	+	0	+	0	0	0	0	0	0	0	+	0	0	0
13	0	0	0	+	0	+	0	0	0	0	+	+	0	0
15	+	0	+	0	0	0	0	0	0	0	0	0	0	0
16	0	0	+	0	0	0	0	0	0	0	0	0	0	0
23	+	0	+	+	+	+	0	0	0	0	0	0	+	+
32	+	0	+	+	0	0	0	0	0	0	0	0	0	0
Total	8	1	10	6	4	3	0	1	1	1	6	1	3	2
Places with either adults or larvae....	8		11		5		1		1		6		3	

In many families reporting suspected cases of yellow fever during the present epidemic, apparently only one, or, at most, two individuals had attacks. The one outstanding exception to this general rule was the Vago family, living on the Serra da Bôa Vista, which reported seven cases with three deaths during January and February. Special studies made about this home to determine, if possible, wherein its mosquito fauna might differ from that of other parts of the infected area, were rewarded by the discovery of millions * of larvae and pupae of *Aëdes scapularis* in a nearby swampy depression. No other focus of similar extent and productivity was found in the entire region during the present study.

Transmission experiments were attempted by Martin Frobisher, Jr., with *Phlebotomi* that had been captured both inside and outside the houses, but great difficulty was encountered in maintaining these insects alive in the laboratory and in inducing them to feed a second time. A few unsuccessful attempts were made to infect monkeys directly with mosquitoes of various species captured at points where cases of yellow fever had recently occurred.

Discussion.

The events leading to the discovery of yellow fever in the Municipio of Santa Thereza in 1932 are almost dramatic in their sequence: the suggestion of the Health Director for the State, in 1930, that this municipio be investigated for the presence of the disease, although he was unable to adduce any concrete basis for such suggestion; the reiteration of this suggestion a year later, when the liver-collection service was being organized; the visit of an experienced field investigator to this municipio and his decision to exclude it from the liver-collection program as "unlikely yellow fever territory"; the declaration of suspected cases; the confirmation of these cases by autopsy and infection of *M. rhesus*.

The discovery of yellow fever in the Valle do Chanaan was to a large extent fortuitous. The local health officer had seen yellow fever during the Rio de Janeiro epidemic of 1928–29, and was familiar with the typical mild cases as well as with the classical picture presented by medical textbooks. Fortunately, he did not know that *Aëdes aegypti* was not prevalent in the Valle do Chanaan. Had the confirmation by autopsy been limited to a single case, certain doubts must have remained in the minds of most students of the disease; but three clinically

* 800 larvae and pupae of *Aëdes scapularis* were counted in 100 cc. of water dipped from this swamp.

typical cases at widely separated points, all positive by microscopical examination, can leave no reasonable doubt of the diagnosis of yellow fever. Moreover, additional confirmation exists in the reproduction of the disease, with typical lesions of yellow fever, in *M. rhesus*, with blood drawn from a patient with a suspected case on the second day of illness.

Had a report of yellow fever, even though supported by pathological diagnosis based on microscopical examination of tissues, been made under similar conditions, previous to the laboratory studies consequent to the establishing of the virus of yellow fever in laboratory animals, it would have been indeed difficult to find any yellow fever epidemiologist willing to accept the possibility of an outbreak of yellow fever in the Valle do Chanaan. Yellow fever was believed to be an urban disease which was transmitted by *Aëdes aegypti*, and was expected to obey the epidemiological rules based on this belief. Even with the mental preparation for rural yellow fever provided by the knowledge that non-domestic species of mosquitoes had proved to be efficient vectors of the virus in the laboratory, the writers found difficulty at first in accepting the diagnosis of yellow fever in the Valle do Chanaan. The failure to find *Aëdes aegypti* in the zone producing suspected cases; the wide scattering of relatively few cases over a large rural area; the absence of suspected cases from the small hamlets in the Valle do Chanaan; the failure to find cases in towns in the surrounding area with high indices of *Aëdes aegypti* breeding, even after the alarm had been given; and the concurrent appearance of cases of both malaria and typhoid in the Valle do Chanaan were all factors which made the epidemiological diagnosis of yellow fever difficult. Although typical clinical cases of yellow fever were observed, any epidemiological investigation unsupported by laboratory methods must have failed to convince investigators that yellow fever was present in the infected area.

There are various indications, including the definite report of a death from this disease, that yellow fever had visited the Valle do Chanaan previously, although absolute proof is, of course, lacking. The age distribution of immunity, as shown by the protection test, tends to confirm this belief, although it might be argued that those of the older age groups had naturally, in the past, had more opportunity for casual infection outside of this area. In any case, the percentage of immune persons found was surprisingly low, considering the distribution of the epidemic and the length of time it was known to have lasted.

It is frankly admitted that the vector or vectors of yellow fever

in the Valle do Chanaan have not been established. The epidemiological findings suggest that the guilty vector or vectors are not house-limited mosquitoes. The evidence points rather to infections occurring in the field, and to the possible transfer of infection from one house to another by the vector, as well as by the human host. The presence of *Phlebotomi* inside the houses as well as outside is significant in the absence of other insects, and studies of the possible rôle of these insects as vectors are needed. The low percentage of immune persons disclosed by mouse-protection tests indicates, we believe, that yellow fever disappears through failure of the insect host, rather than through failure of the human host. This failure of the insect host was probably due to changes in meteorological conditions affecting either its breeding, its flight range and activity, or the length of time necessary for the development of infectivity.

The most logical explanation which can now be given of the epidemiology of yellow fever in the Valle do Chanaan is that the virus of yellow fever is from time to time introduced from nearby silent endemic areas where *Aëdes aegypti* is present. Once introduced, the disease is transmitted by some relatively inefficient but widespread vector.

Another possible hypothesis, which must be considered in the future, is that there may be some long-lived, relatively inefficient vector, possibly seasonal in its activity, capable of transmitting yellow fever. The existence of such a vector would give a satisfactory explanation for continued suspicion of this area, and would also account for the appearance of apparently isolated cases in other areas, not satisfactorily explained on the hypothesis of mosquito transmission. It should be remembered that the State Health Department had vague suspicions regarding yellow fever in this area more than a year before the appearance of the first case now definitely considered to have been yellow fever. The intervening winter temperatures of the Valle do Chanaan are believed to be sufficiently low to have prevented the persistence of the virus in this area on the basis of mosquito transmission.

In considering the rôle of the insect vector in the epidemiology of yellow fever, it must be remembered that in the laboratory wide variations have been found in the ability of different species to transmit the disease. Certain species, the outstanding example of which is *Aëdes aegypti*, seem to show a high percentage of exposed mosquitoes infective after an incubation period of ten to twelve days at ordinary tropical temperatures. Apparently, some species are less efficient as vectors, only a small percentage of the mosquitoes effecting transmission.

Other species apparently require a longer period of incubation before becoming infective, and still others have not been tested because of their refusal to take repeated feedings in the laboratory. In considering any insect as a possible vector of yellow fever, thought must be given to its breeding and feeding habits as well as to its actual ability to transmit the disease in the laboratory. From a consideration of published laboratory experiments on the ability of various species of mosquitoes to transmit yellow fever, and of the results of adult captures and larval collections at many points in the Valle do Chanaan, it is believed that *Aëdes scapularis* was the most probable vector among the various mosquitoes captured and studied. This mosquito was widespread and proved to be a vicious biter. However, the possibility that *Aëdes fluviatilis* may have been in part responsible, cannot be entirely discounted, since it is apparently a more efficient vector in the laboratory than is *Aëdes scapularis*. Many parts of the Valle do Chanaan possess suitable natural breeding places for *A. fluviatilis*, although but few adults were captured with animal bait. The finding of this species in ant-rings (Soper and Serafim 17) indicates that it may be a facultative artificial deposit breeder.

Various species of *Anopheles* and *Culex* were found in abundance in the Valle do Chanaan, but they were not given serious consideration as possible vectors, since all previously published reports indicated that species of these genera had not been incriminated. Kerr (16) has recently shown that *Culex thalassius* may become infective after an incubation period of more than double the usual period required by *Aëdes aegypti*.* However, any factor which prolongs the period of incubation in the insect host, will, of necessity, greatly reduce the efficiency of that host as a vector, since the mortality of mosquitoes in nature is known to be very high.

In considering the possibility of intermediate vectors, other than mosquitoes, only *Phlebotomi* were found in sufficient numbers and so distributed as to be open to suspicion. Further laboratory tests are needed to determine the relationship of this insect to yellow fever transmission.

As a result of the observations in the Valle do Chanaan, certain conceptions of the epidemiology of yellow fever will have to be revised. Natural infections are not always acquired by the bite of an infected *Aëdes aegypti*, and it has been shown that a local epidemic of several months' duration may be maintained without the intervention of this

* N. C. Davis has recently succeeded in transmitting yellow fever by the bite of *Culex* (*Culex*) *quinquefasciatus* (Say), and *Psorophora* (*Janthinosoma*) *ferox* (Humboldt). (Unpublished communication.)

species. Yellow fever is not necessarily an urban disease. Villages, whose populations are largely non-immune, may have no outbreaks of the disease, though situated in infected rural areas. Yellow fever is not necessarily a house disease, nor is it necessarily transmitted by house mosquitoes. It may persist in a rural community for months and disappear spontaneously, through some failure of the intermediate host, leaving a large percentage of the local population non-immune. Although spontaneous disappearance apparently occurred in the Valle do Chanaan, it has been shown that an entirely rural epidemic, not transmitted by *Aëdes aegypti*, did maintain itself for at least three months; therefore the possibility of regional rural endemicity must be considered. It must also be emphasized that this slowly burning rural epidemic disappeared spontaneously through no failure of the human host. How long an epidemic of this type may maintain itself under more favorable meteorological conditions cannot be stated. The possibility of a long-continued rural regional endemicity, more or less independent of movement of population, must not be overlooked in the future. One of the most surprising findings of the present study is the low percentage of positive protection tests in combination with the widespread dissemination of the disease. May it not be possible that a slowly burning epidemic of this type is more serious from the standpoint of final elimination of yellow fever from an area, than is the rapidly explosive type, which burns itself out quickly and disappears from failure of the human host?

The finding of yellow fever without *Aëdes aegypti* comes as a distinct disappointment to those workers who, in the face of repeated laboratory demonstrations of the infectibility of other species, took consolation in the words of Carter (10). After admitting the possibility of other vectors being found for yellow fever, Carter stated:

> Owing to the invariable disappearance of yellow fever when this species (*Aëdes aegypti*)—and this species alone—is sufficiently controlled, it seems quite certain that in the Americas no other mosquito associated closely with man is a vector.

But, in the case of a widespread rural epidemic in which the transmitting agent is not a house mosquito breeding in artificial containers, the accepted methods of yellow fever prophylaxis, so universally and so dramatically successful for three decades, are not applicable. Possibly other methods, similar to those already used in combating malaria, may be adapted to the control of the rural vectors of yellow fever, once these have been identified and their habits studied. Should further investigation reveal large rural endemic areas, little hope is entertained for the development of economically feasible prophylactic methods.

From the point of view of the general problem of yellow fever control, it is impossible to estimate the importance of the present findings. Generalizations must be very guarded, because the conditions in the Valle do Chanaan are believed to be exceptional, at least, for the yellow fever zone in Brazil. This epidemic was apparently self-limited, and it is possible that there are no rural regions in America which are truly endemic for yellow fever. Future studies of the distribution of yellow fever should include entomological surveys, and protection tests with the sera of persons born and residing constantly in rural areas, as well as routine examination of liver tissue from all persons dying in suspected areas after a fever of short duration.

Summary and conclusions.

Yellow fever was discovered in the Valle do Chanaan, Municipio of Santa Thereza, Espirito Santo, Brazil, early in March, 1932. This finding was confirmed by examination of tissues from three persons dying of the disease, and by infection of *M. rhesus* with blood from an early case. Field studies indicated that:

1. Yellow fever was, during at least three months, widespread in a strictly rural district in which *Aëdes aegypti* was not found, even after a thorough and prolonged search begun six weeks before the apparently spontaneous disappearance of the disease from this district.

2. Of the species of mosquito incriminated by laboratory experiments as potential vectors of the yellow fever virus, only *Aëdes* (*Ochlerotatus*) *scapularis*, Rondani, and *Aëdes* (*Taeniorhynchus*) *fluviatilis*, Lutz, exist throughout the infected area in sufficient numbers to merit consideration as being possibly responsible for the epidemic. Of these, *Aëdes scapularis* was found much more frequently, both as larva and as adult, and is believed to be the more dangerous species.

3. Despite this epidemic and despite rumors of previous invasions of yellow fever in the Valle do Chanaan, immunity to the disease, as indicated by the mouse-protection test on sera from several hundred residents, widely disseminated geographically, was limited to a surprisingly low percentage of those tested. On the basis of this low percentage of immune persons, the spontaneous disappearance of yellow fever in this area is attributed to inefficiency of the insect vector, rather than to failure of the human host.

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